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Effects of Various Fluoride Solutions on Enamel Erosion in vitro

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Key Words
Enamel · Erosion · Fluoride

Abstract
The objective of this in vitro study was to investigate the effect of different fluoride solutions on enamel erosion. Human enamel specimens were pretreated with 1 of 10 different fluoride solutions (n = 20): TiF_4, NaF, AmF, ZnF_2, or SnF_2, each at native pH (pH range: 1.2–7.8) or buffered pH (pH = 4). The control group samples received no fluoride pretreatment. All samples were then eroded by citric acid (pH 2.6) for 6 × 1 min daily over 5 days. Between the erosive cycles, the samples were stored in artificial saliva. Erosion effects were investigated by surface profilometry (n = 10), scanning electron microscopy (n = 4), and energy-dispersive X-ray spectroscopy (n = 6) after fluoride pretreatment and after erosion. To test the effects of pH only, additional experiments were carried out with fluoride-free solutions at similar pH to that of fluoride solutions. In general, AmF solution was more effective in protecting enamel erosion compared to all other fluoride agents. However, the application of native TiF_4, native and buffered SnF_2, and native and buffered AmF solutions also resulted in significantly less enamel loss compared to the control group. A Ti-rich coating was formed after application of native TiF_4, but partially dissolved due to erosive attack. Samples pretreated with SnF_2 showed a significant increase in surface tin content. Surface fluoride concentration was significantly increased by native TiF_4, native and buffered AmF, buffered ZnF_2, and buffered NaF application. Under the current experimental setting, the fluoride agents at lower pH had better protective potential. Highly concentrated TiF_4, AmF, and SnF_2 solution was effective in inhibiting erosion of enamel.

Dental erosion, which is defined as the loss of tooth substance by chemical processes not involving bacteria, is becoming an increasingly important factor when considering long-term dental health [Lussi et al., 2004]. Considering the presence of dental erosion has been growing steadily in the last few decades [Jaeggi and Lussi, 2006], interceptive methods to prevent dental erosion are needed.

Several methods have been proposed to prevent the progression of dental erosion, such as fluoride application and modification of acidic beverages [Magalhaes et al., 2009]. Although the mechanism of fluoride application in prevention of dental erosion is still a matter of controversy, many studies have shown the potential protective effects of fluoride application on dental erosion [Ganss et

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al., 2004; Lagerweij et al., 2006]. Interestingly, most of the studies were done with fluoride agents that have shown their anti-caries effects before, such as sodium fluoride (NaF), amine fluoride (AmF), stannous fluoride (SnF_2) and zinc fluoride (ZnF_2). It was reported that SnF_2 application led to a much greater reduction of mineral loss compared to NaF and AmF [Ganss et al., 2008]. In an in vitro study investigating titanium tetrafluoride (TiF_4), SnF_2, and NaF, all fluoride agents showed protective effects on erosion while TiF_4 protected the enamel surface almost completely [Hove et al., 2006]. With regard to TiF_4, several studies have shown that it had a great inhibitory effect against dental erosion [Hove et al., 2006; Schlueter et al., 2007; Wiegand et al., 2008], while other studies found that TiF_4 reduced erosion only to a small extent compared to the control group [Vieira et al., 2005; Magalhaes et al., 2008]. Furthermore, it has been pointed out that the efficacy of fluoride agents at native and buffered pH in protecting enamel erosion is distinctly different [Arnold et al., 2007; Wiegand et al., 2009b].

In short, the fluoride agents showed a wide range in their efficacy against erosion in diverse experimental designs. Thus, a systematic investigation of pH- and concentration-controlled fluoride preparations on erosion is necessary in order to get better understanding of the effects of different fluoride compounds on erosion. However, relatively little information is available to date.

Therefore, the objective of this study was to evaluate the effects of different fluoride solutions on human enamel using surface profilometry, scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDS). Two hypotheses were proposed: (1) different fluoride agents have a different protective effect on human enamel erosion, and (2) fluoride agents at their native and a specific buffered pH differ in their protective ability against human enamel erosion.

Materials and Methods

Study Design

Enamel samples were pretreated with distilled water (negative control) or 1 of 10 different fluoride solutions (TiF_4, SnF_2, AmF, ZnF_2, NaF, each at native pH or pH 4, each n = 20) and subjected to a five-day cyclic de- and remineralization procedure. Demineralization was performed with citric acid at pH 2.6 for 6 × 1 min daily and the specimens were immersed in artificial saliva during the remaining time. The effects of fluoride treatment and erosion were analyzed using surface profilometry (n = 10), SEM (n = 4), and EDS (n = 6). Additionally, to test the effects of pH alone on erosion, enamel specimens were pretreated with four fluoride-free buffer solutions at pH 1.2, 2.7, 4 and 7.8, respectively, analogous to the pH of the fluoride solutions used in this study. Subsequently, the samples were subjected to the same cyclic de- and remineralization procedure and analyses as described above.

Specimen Preparation

Three hundred enamel samples (3 mm in diameter) were obtained from the labial and palatal surfaces of 150 previously extracted, caries-free human molar teeth. The samples were embedded in ring-shaped ceramic moulds (3 mm diameter, 3 mm thickness) with acrylic resin (Paladur, Heraeus Kulzer, Wehrheim, Germany). The ceramic moulds were cut from a ceramic tube (Degussit, Friatec/Degussa, Düsseldorf, Germany) using a water-cooled low speed saw (Isomet, Buehler, Lake Bluff, Ill., USA). The embedded specimens were ground flat and polished with water-cooled carborundum discs [1,200, 2,400 and 4,000 grit (FEPA-P), Water proof silicon carbide paper, Stuers, Erkrat, Germany]. This procedure resulted in the removal of about 200 μm depth of enamel, which was controlled with a digital micrometer (Holex, Nuremberg, Germany). The polished specimens were cleaned in distilled water in an ultrasonic cleaner (M. Scherrer, Wil, Switzerland) for 1 min to remove any debris. Before use, all the specimens were stored in 100% humidity.

Fluoride Solution Pretreatment

Each of the experimental groups were pretreated with one of the fluoride solutions as follows: native TiF_4 solution (0.48 M F, pH 1.2), buffered TiF_4 solution (0.48 M F, pH 4.0), native SnF_2 solution (0.48 M F, pH 2.7), buffered SnF_2 solution (0.48 M F, pH 4.0), native AmF solution (0.48 M F, pH 4.6), buffered AmF solution (0.48 M F, pH 4.0), native ZnF_2 solution (0.20 M F, pH 5.1), buffered ZnF_2 solution (0.20 M F, pH 4.0), native NaF solution (0.48 M F, pH 7.8), buffered NaF solution (0.48 M F, pH 4.0). All the solutions were prepared freshly prior to application to the specimens. The specimens were separately fixed in plastic chambers. The chambers were then filled with 5 ml of the respective fluoride solution and left undisturbed for 3 min at room temperature. After treatment, all the samples were rinsed with distilled water for 30 s. Specimens of the control group were treated with distilled water for 3 min. For the additional experiment regarding the effect of pH of pretreatment solution on enamel erosion alone, specimens were pretreated with fluoride-free solutions at different pH (pH 1.2, 2.7, 4 and 7.8; n = 20) for 3 min. Then, the samples were rinsed in the same manner as mentioned above. All the details of fluoride solutions and fluoride-free buffer solutions used are shown in table 1.

Cyclic Erosive Treatment

Following the fluoride pretreatment, which was performed one time only, the cycling de- and remineralization regimen was performed 6 times daily for 5 days. The samples were first eroded by immersion in 5 ml citric acid (pH 2.6) for 1 min. After erosion, the samples were rinsed with distilled water for 30 s and stored for 1 h in 5 ml artificial saliva until the next erosion challenge. The artificial saliva was mixed according to the formulation given by Klimek et al. [1982]. 500 ml of artificial saliva contained 0.001 g ascorbic acid, 0.015 g glucose, 0.290 g NaCl, 0.085 g $CaCl_2$, 0.080 g NH_4Cl, 0.635 g KCl, 0.080 g NaSCN, 0.165 g KH_2PO_4, 0.100 g carbamide, 1.350 g mucin and 0.170 g Na_2PO_4. After 6 daily cycles, specimens were stored in artificial saliva overnight. Specimens of the control group were maintained in artificial saliva for the entire experimental period. The artificial saliva was renewed every day.

Table 1. Contents and pH of the solutions used in this study

Solutions		Contents in 100 ml solution	pH
TiF_4	Native	1.50 g titanium tetrafluoride	1.2
	Buffered	1.50 g titanium tetrafluoride, 2.45 g sodium citrate	4.0
SnF_2	Native	3.75 g stannous fluoride	2.7
	Buffered	3.75 g stannous fluoride, 2.50 g sodium citrate	4.0
AmF	Native	65.10 g Olaflur solution	4.6
	Buffered	65.10 g Olaflur solution, 4.25 ml 5 M phosphoric acid	4.0
ZnF_2	Native	1.60 g zinc fluoride tetrahydrate	5.1
	Buffered	1.60 g zinc fluoride tetrahydrate, 0.30 ml 5 M phosphoric acid	4.0
NaF	Native	2.02 g sodium fluoride	7.8
	Buffered	2.02 g sodium fluoride, 7.50 ml 5 M phosphoric acid	4.0
Flouride-free buffer	Solution 1	9.00 ml 0.1 M disodium citrate, 91.00 ml 0.1 M hydrogen chloride	1.2
	Solution 2	36.00 ml 0.1 M disodium citrate, 64.00 ml 0.1 M hydrogen chloride	2.7
	Solution 3	55.10 ml 0.1 M disodium citrate, 44.90 ml 0.1 M hydrogen chloride	4.0
	Solution 4	7.50 ml 0.07 M potassium dihydrogen phosphate, 92.50 ml 0.07 M disodium hydrogen phosphate	7.8

Profilometric Analysis

Enamel loss was measured by a stylus profilometer (Perthometer S2/GD 25, Mahr, Göttingen, Germany) after fluoride pretreatment and after erosion. The procedure has been described in detail previously [Yu et al., 2009]. Three profiles were performed on each specimen via scanning from the reference (ceramic mould) surface to the treated surface. Profiles before and after treatment were exactly matched using a custom-designed software (4D client) so that the difference between the profiles could be determined. An average of these three readings (μm) was obtained and used for data analysis.

SEM and EDS

After fluoride pretreatment, four samples from each group were randomly selected for SEM observation. The specimens were mounted on aluminum stubs and sputter coated with platinum, and then examined using a Supra 50 VP Scanning Electron Microscope (Carl Zeiss NTS, Oberkochen, Germany) with an acceleration voltage of 2 kV. Likewise, after erosion, four samples from each group were selected and observed under SEM. Furthermore, quantitative changes of the surface composition of the fluoride-treated and eroded samples were evaluated using EDS. The EDS analysis was performed with the same SEM equipped with an EDAX PV7715/89 ME energy-dispersive X-ray spectrometer. Six specimens from each group were sputter coated with carbon and the respective EDS spectra were obtained in a 200 × 200 μm area with an acceleration voltage of 15 kV. The spectra were later analyzed using the EDAX Genesis Spectrum software package (EDAX, Mahwah, N.J., USA).

Statistical Analysis

The data were analyzed using the SPSS statistical software package (SPSS 13.0 for Windows, SPSS, Chicago, Ill., USA). The Kolmogorov-Smirnov test was used for checking the normal distribution of the data. The results of profilometric analysis and EDS analysis were analyzed by one-way analysis of variance (ANOVA) followed by Tukey multiple comparison tests. Since data for F content (after pretreatment and after erosion) and P content after erosion were not normally distributed, Mann-Whitney test was performed for comparison of respective contents in fluoride-treated groups and the control group. The level of significance was set at $p < 0.05$.

Results

Profilometric Analysis

Table 2 shows the enamel loss in different groups after fluoride application and after erosive attack. After fluoride treatment, the native TiF_4-treated samples showed the most pronounced enamel loss (0.25 μm), although no significant differences were found among all groups including the control group (distilled water). In most cases, all fluoride applications reduced erosive substance loss. The application of AmF at pH 4 resulted in almost no erosive loss (97% reduction compared to controls), while the other fluoride group led to reduction of enamel loss from 15 to 82% compared to the control. However, only the application of native TiF_4, native and buffered SnF_2, and native and buffered AmF was able to significantly decrease erosive enamel loss compared to the control group. With regard to the total loss due to fluoride treatment and erosive attack, similar results were found. Total erosive substance loss was significantly smaller for TiF_4 application at native pH than at the buffered pH. All other fluoride solutions at lower pH reduced the enamel loss, but not significantly, compared with the respective solutions at higher pH.

Table 2. Means (standard deviations) of substance loss (in μm) for the fluoride-treated groups and control group

Groups	pH		Substance loss after pretreatment		Substance loss after erosion		Total substance los	
	native	buffered	native pH	buffered pH	native pH	buffered pH	native pH	buffered pH
TiF_4	1.2	4.0	0.25 (0.15)[a]	0.15 (0.22)[a]	1.03 (0.35)[a]	2.19 (0.48)[b]	1.28 (0.36)[a, b]	2.34 (0.38)[c]
SnF_2	2.7	4.0	0.12 (0.13)[a]	0.01 (0.10)[a]	0.72 (0.45)[a]	0.96 (0.39)[a]	0.84 (0.54)[a, b]	0.96 (0.34)[a]
AmF	4.6	4.0	–0.13 (0.09)[a]	0.09 (0.03)[a]	0.40 (0.19)[a]	0.08 (0.27)[a]	0.17 (0.32)[b]	0.16 (0.30)[b]
ZnF_2	5.1	4.0	0.00 (0.09)[a]	0.16 (0.03)[a]	2.38 (0.17)[b]	1.94 (0.62)[b]	2.38 (0.08)[c]	2.09 (0.60)[c]
NaF	7.8	4.0	–0.07 (0.13)[a]	–0.07 (0.21)[a]	2.42 (0.29)[b]	2.08 (0.15)[b]	2.35 (0.35)[c]	2.01 (0.34)[c]
Distilled water	–	–	0.07 (0.05)[a]	–	2.88 (0.53)[b]	–	2.96 (0.55)[c]	–

Positive values indicate substance loss of the respective group. Comparisons of substance loss are made between all groups within the same column (pretreatment, erosion and total). Within the same column, values marked with the same letter were not significantly different.

Table 3. Means (standard deviations) of substance loss (in μm) for the buffer-treated groups and control group

Groups	pH	Substance loss after pretreatment	Substance loss after erosion	Total substance loss
Distilled water	–	0.07 (0.05)[a]	2.88 (0.53)[a]	2.96 (0.55)[a]
Fluoride-free buffer 1	1.2	3.17 (0.32)[b]	2.93 (0.75)[a]	6.70 (0.70)[b]
Fluoride-free buffer 2	2.6	0.52 (0.15)[c]	2.28 (0.56)[a]	2.53 (0.63)[a]
Fluoride-free buffer 3	4.0	0.32 (0.11)[a, c]	2.24 (0.47)[a]	2.49 (0.61)[a]
Fluoride-free buffer 4	7.8	0.11 (0.13)[a]	2.80 (0.65)[a]	2.62 (0.43)[a]

Positive values indicate substance loss of the respective group. Comparisons of substance loss are made between all groups within the same column (pretreatment, erosion and total). Within the same column, values marked with the same letter were not significantly different.

For the samples pretreated with fluoride-free solutions (table 3), the application of the solution at pH 1.2 and pH 2.6 led to significantly higher erosive enamel loss compared with the ones at pH 4 and pH 7.8, as well as the control specimens treated with distilled water. However, no significant differences were found in substance loss due to erosion among these four groups and the control group.

SEM and EDS

Representative SEM images and EDS results are shown in figures 1–6. The application of native TiF_4, native and buffered AmF, and buffered NaF resulted in distinct alteration in the surface morphology of human enamel. After application of native TiF_4, a surface layer with some globular materials was formed on the enamel surface. This layer was found to have some microcracking and spacing (fig. 1a). The underlying enamel was extremely porous and granular in appearance (fig. 1b). After erosion, the coating was partially disappeared and the rest of the surface demonstrated severe etching (fig. 1e). The morphologic appearance of the enamel surfaces revealed the presence of precipitates in both native and buffered AmF-treated samples. The precipitates varied in appearance. The globules on the native AmF-treated specimens were larger than the ones on the buffered AmF-treated specimens. However, similar surface morphology with distinctly larger globules was found in both AmF-treated samples after 30 min erosion (fig. 2). The globular precipitation was also found on the buffered NaF-treated samples, but was completely dissolved after erosion (fig. 3). SnF_2 treatment did not produce globular deposits on the enamel surface. The native SnF_2-treated surfaces revealed small pits, while larger pores were found on the surface after erosion (fig. 4a, b). Although no visible difference was found after treatment with buffered SnF_2, moderate demineralized surface with some pores were found after erosion (fig. 4c, d). Apart from the phenomenon men-

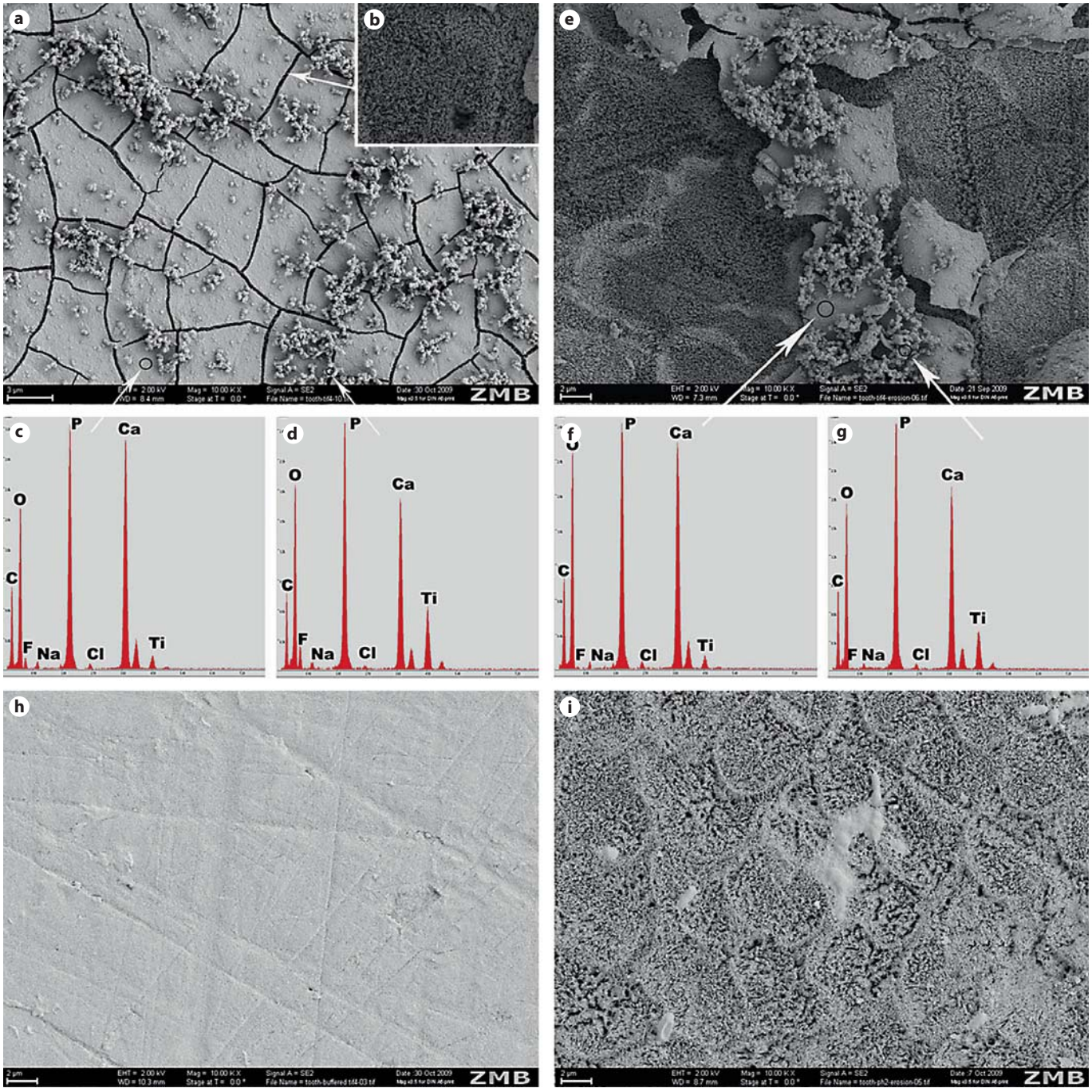

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Fig. 1. Representative images of enamel treated with native TiF_4 (pH 1.2). **a** SEM images of enamel surface after native TiF_4 application. ×10,000. **b** SEM images of underlying enamel. ×80,000. **c** EDS spectra of the surface layer. **d** EDS spectra of the globular materials. **e** SEM images of native TiF_4-treated enamel surface after erosion. ×10,000×. **f** EDS spectra of the surface layer after erosion. **g** EDS spectra of the surface globules after erosion. **h** SEM images of enamel surface after buffered TiF_4 (pH 4) application. ×10,000. **i** SEM images of buffered TiF_4-treated enamel surface after erosion. ×10,000.

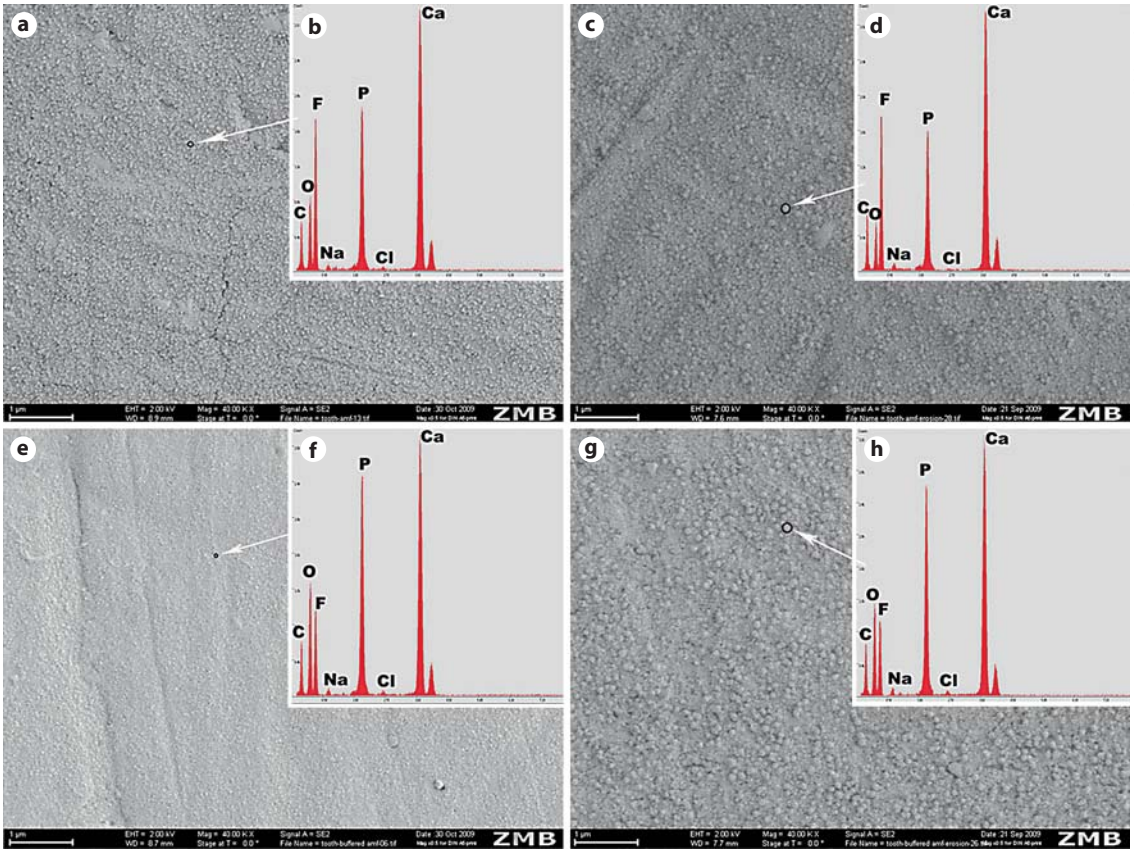

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Fig. 2. Representative images of enamel treated with AmF. **a** SEM image of enamel surface after native AmF (pH 4.6) application. ×40,000. **b** EDS spectra of surface globules after native AmF application. **c** SEM images of native AmF-treated enamel surface after erosion. ×40,000. **d** EDS spectra of surface globules after erosion. **e** SEM image of enamel surface after buffered AmF (pH 4) application. ×40,000. **f** EDS spectra of surface globules after buffered AmF application. **g** SEM images of buffered AmF-treated enamel surface after erosion. ×40,000. **h** EDS spectra of surface globules after erosion.

tioned above, the sample surfaces appeared smooth and structureless after distilled water or fluoride application (ZnF_2 and native NaF), while the severely etched surfaces were found after erosive challenges (fig. 5 and 6).

Table 4 presents the concentration (wt%) of the respective elements (given that some of the data were not normally distributed, medians and inter-quartile ranges were used to present the data). All the eroded samples showed a decrease in surface Ca concentration, although some of the differences were not statistically significant. Treatment with native TiF_4 led to a significant increase in Ti compared to treatment with buffered TiF_4. Likewise, a larger amount of Sn was found after applying native SnF_2. The concentration of these metal ions (Ti and Sn) was reduced after erosion. The application of native TiF_4, native and buffered AmF, buffered ZnF_2, and buffered NaF resulted in a significant increase in F, which was significantly higher for native AmF than for the other solutions. After erosion, a decrease was found in F concentration accompanying with an increase in C concentration.

All the samples pretreated with fluoride-free solutions at different pH showed a similar surface tomography and composition as distilled water after 30 min erosive attack (table 5).

Discussion

Based on the above results, the hypotheses that different fluoride agents have a different effect on human enamel erosion and that the pH of fluoride agents affects

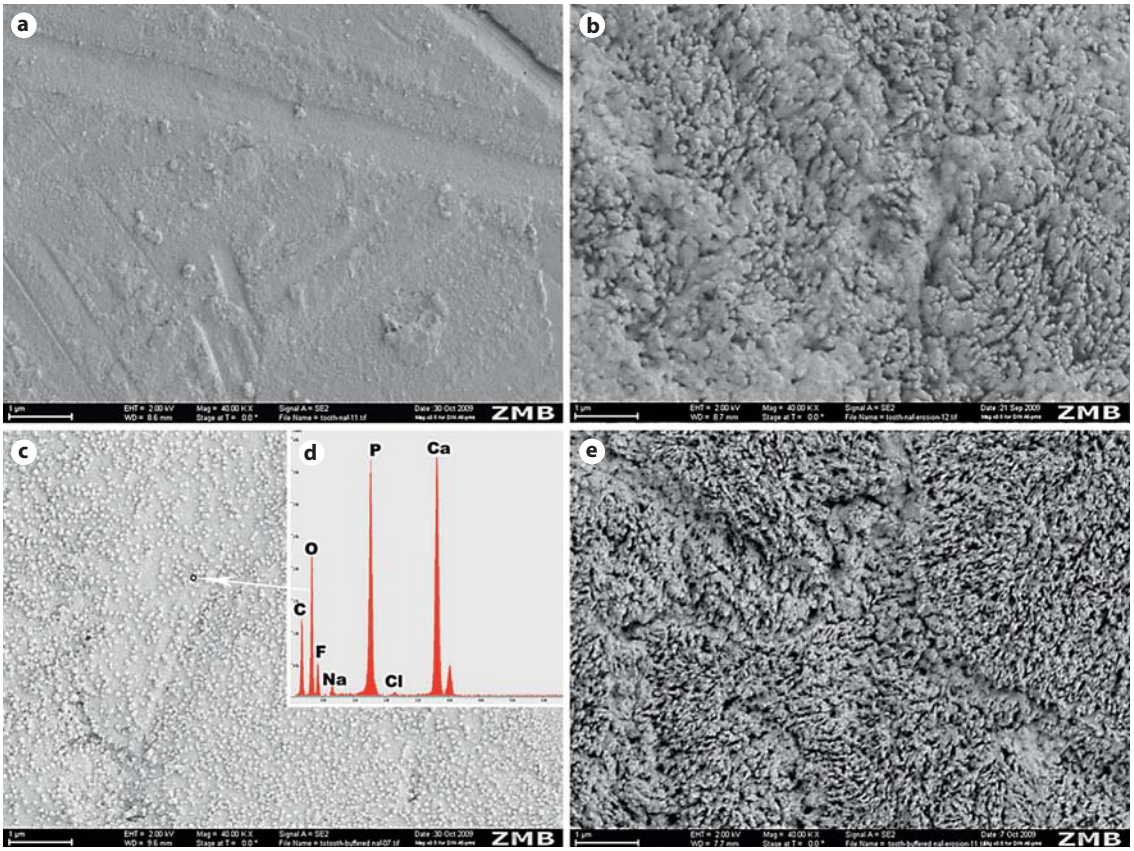

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Fig. 3. Representative images of enamel treated with NaF. **a** SEM image of enamel surface after native NaF (pH 7.8) application. ×40,000. **b** SEM image of native NaF-treated enamel surface after erosion. ×40,000. **c** SEM image of enamel surface after buffered NaF (pH 4) application. ×40,000. **d** EDS spectra of surface globules after buffered NaF application (arrow marks the corresponding surface area). **e** SEM image of buffered NaF-treated enamel surface after erosion. ×40,000.

enamel erosion under the testing conditions were therefore accepted.

In the literature, many techniques have been used to investigate the effects of erosive attacks on dental hard tissues. Surface profilometry, microradiography, chemical analysis, micro-indentation, and SEM were considered the most established laboratory assessment in evaluating enamel erosion [Barbour and Rees, 2004; Attin, 2006]. In the present study, surface profilometry and SEM were selected to analyze the mechanical effect of the fluoride solutions on enamel erosion in order to measure substance loss accurately and provide visual information on surface precipitates and change of the surface morphology. On the other hand, EDS provides a specific method to determine the concentration of chemical elements on substratum surfaces, being largely used in engineering and chemistry, but not in many studies in dentistry [Paradella et al., 2008]. Since the detection limit of EDS is about 0.1 wt% [Kuisma-Kursula, 2000], EDS seems to be a sufficient tool to evaluate the chemical changes of fluoridation and erosion on the enamel surface.

The profilometer system used in this study had a detection limit of 0.105 μm and high reproducibility [Attin et al., 2009]. Since the force applied by the contact stylus (about 0.7 mN during the measurement) was quite slight and the subsurface changes in mineral content are relatively small, profilometer of this type is considered appropriate for evaluation of enamel erosion [Ganss et al., 2009].

In general, AmF solution was more effective in protecting enamel erosion compared to other fluoride solutions. In accordance with a previous study [Rosin-Grget et al.,

Table 4. Medians and inter-quartile ranges (25th percentiles/75th percentiles) of concentration (wt%) of the respective elements in fluoride-treated groups and control group by EDS analysis (200 × 200 μm)

Group	pH	After pretreatment						After erosion					
		C	F	P	Ca	Sn	Ti	C	F	P	Ca	Sn	Ti
Native TiF_4	1.2	14.42 (14.22/ 14.59)[a, b]	2.26 (2.05/ 2.51)[a]	14.89 (14.79/ 14.99)[a]	27.31 (26.73/ 27.53)[a]	–	3.18 (2.94/ 3.31)[a]	19.81 (18.08/ 21.57)[a]	0.40 (0.31/ 0.52)[a]	14.63 (13.77/ 15.48)[a, b]	26.32 (23.60/ 30.71)[a]	–	1.46 (1.06/ 2.03)[a]
Buffered TiF_4	4.0	14.57 (14.48/ 14.77)[a]	0.39 (0.31/ 0.47)[b]	15.24 (14.75/ 15.62)[a]	30.64 (29.96/ 31.58)[b]	–	0.27 (0.13/ 0.44)[b]	20.43 (20.40/ 20.63)[a]	0.27 (0.15/ 0.49)[a]	15.54 (14.62/ 15.83)[a]	28.51 (27.20/ 28.55)[a, b]	–	–
Native SnF_2	2.7	14.64 (14.42/ 14.77)[a]	0.32 (0.22/ 0.41)[b]	15.32 (15.19/ 15.48)[a]	30.77 (30.32/ 31.39)[b]	2.09 (1.92/ 2.26)[a]	–	19.46 (18.38/ 20.76)[a]	–	15.14 (14.77/ 15.48)[a]	27.89 (26.11/ 29.73)[a, b]	1.06 (0.94/ 1.16)[a]	–
Buffered SnF_2	4.0	14.19 (13.91/ 14.43)[a, b]	0.22 (0.10/ 0.30)[b]	15.35 (15.12/ 15.54)[a]	30.37 (30.04/ 30.68)[b]	1.26 (1.14/ 1.36)[b]	–	17.49 (17.21/ 17.69)[a]	0.29 (0.25/ 0.40)[a]	14.91 (14.55/ 15.89)[a]	27.97 (27.34/ 28.77)[a, b]	1.08 (0.99/ 1.19)[a]	–
Native AmF	4.6	14.68 (14.49/ 15.44)[a]	26.24 (25.67/ 26.24)[c]	10.46 (10.02/ 10.89)[b]	32.84 (32.40/ 33.01)[c]	–	–	20.92 (18.18/ 25.58)[a]	8.94 (0.38/ 23.04)[a, b]	12.56 (8.64/ 14.62)[b]	30.38 (27.15/ 32.01)[b]	–	–
Buffered AmF	4.0	14.75 (14.23/ 14.89)[a]	16.56 (15.20/ 17.12)[d]	13.19 (12.62/ 13.25)[c]	32.21 (31.65/ 32.75)[c, d]	–	–	17.35 (16.87/ 17.70)[a]	16.13 (14.37/ 18.40)[b]	13.33 (12.99/ 13.54)[a, b]	30.53 (29.70/ 31.69)[b]	–	–
Native ZnF_2	5.1	13.83 (13.38/ 13.94)[b]	0.25 (0.15/ 0.44)[b]	15.49 (15.18/ 15.79)[a]	31.41 (30.90/ 31.77)[b]	–	–	19.98 (19.81/ 20.29)[a]	0.10 (0.08/ 0.16)[a]	14.59 (14.55/ 14.64)[b]	28.32 (28.24/ 28.36)[a, b]	–	–
Buffered ZnF_2	4.0	14.49 (14.38/ 14.69)[a, b]	3.40 (3.07/ 3.71)[e]	15.30 (15.15/ 15.39)[a]	32.08 (31.95/ 32.68)[c, d]	–	–	18.09 (16.63/ 19.88)[a]	0.31 (0.08/ 0.34)[a]	15.32 (14.61/ 15.73)[a]	28.46 (26.15/ 30.11)[a, b]	–	–
Native NaF	7.8	14.12 (13.88/ 14.39)[a, b]	0.88 (0.56/ 1.03)[b]	15.38 (15.19/ 15.68)[a]	31.51 (31.10/ 31.87)[b, c]	–	–	20.08 (19.12/ 20.29)[a]	0.10 (0.06/ 0.21)[a]	15.15 (14.45/ 15.51)[a]	29.51 (28.41/ 30.58)[a, b]	–	–
Buffered NaF	4.0	14.08 (13.99/ 14.50)[a, b]	5.82 (4.45/ 5.91)[f]	15.19 (14.96/ 15.35)[a]	32.08 (31.47/ 32.68)[c, d]	–	–	19.27 (19.10/ 19.71)[a]	1.04 (0.84/ 1.16)[a]	13.19 (12.80/ 13.58)[a, b]	28.78 (28.48/ 29.06)[a, b]	–	–
Distilled water	–	14.04 (13.90/ 14.24)[a, b]	0.07 (0.03/ 0.08)[b]	15.46 (15.15/ 15.88)[a]	31.00 (30.02/ 32.36)[b, d]	–	–	20.33 (20.22/ 20.54)[a]	0.13 (0.09/ 0.16)[a]	14.15 (13.92/ 14.25)[a, b]	27.59 (27.04/ 27.90)[a, b]	–	–

Within the same column, values marked with same letter were not significantly different. – = The concentration of the respective element was below the detection limit.

Table 5. Mean (standard deviation) of concentration (wt%) of the respective elements in buffer-treated groups and control group by EDS analysis (200 × 200 μm)

Groups	pH	After pretreatment				After erosion			
		C	F	P	Ca	C	F	P	Ca
Distilled water	–	14.06 (0.19)[a]	0.06 (0.03)[a]	15.50 (0.38)[a]	31.03 (1.26)[a]	20.36 (0.17)[a]	0.13 (0.04)[a]	14.11 (0.18)[a]	27.51 (0.46)[a]
Buffer solution 1	1.2	17.18 (0.22)[b]	0.06 (0.07)[a]	13.31 (0.15)[b]	26.97 (0.45)[b]	18.68 (0.22)[a]	0.06 (0.06)[a]	14.34 (0.14)[a]	28.16 (0.41)[a]
Buffer solution 2	2.7	16.88 (1.29)[b]	0.12 (0.09)[a]	14.98 (0.48)[b]	30.97 (1.29)[a]	18.12 (0.33)[a]	0.08 (0.02)[a]	14.13 (0.20)[a]	28.24 (0.33)[a]
Buffer solution 3	4.0	15.26 (1.23)[a, b]	0.06 (0.05)[a]	14.94 (0.33)[a]	30.25 (0.72)[a]	19.32 (0.80)[a]	0.09 (0.02)[a]	14.78 (0.08)[a]	28.13 (0.49)[a]
Buffer solution 4	7.8	13.78 (0.12)[a]	0.10 (0.04)[a]	15.86 (0.05)[a]	32.23 (1.15)[a]	18.84 (0.17)[a]	0.09 (0.01)[a]	14.97 (0.05)[a]	28.97 (0.29)[a]

Within the same column, values marked with same letter were not significantly different.

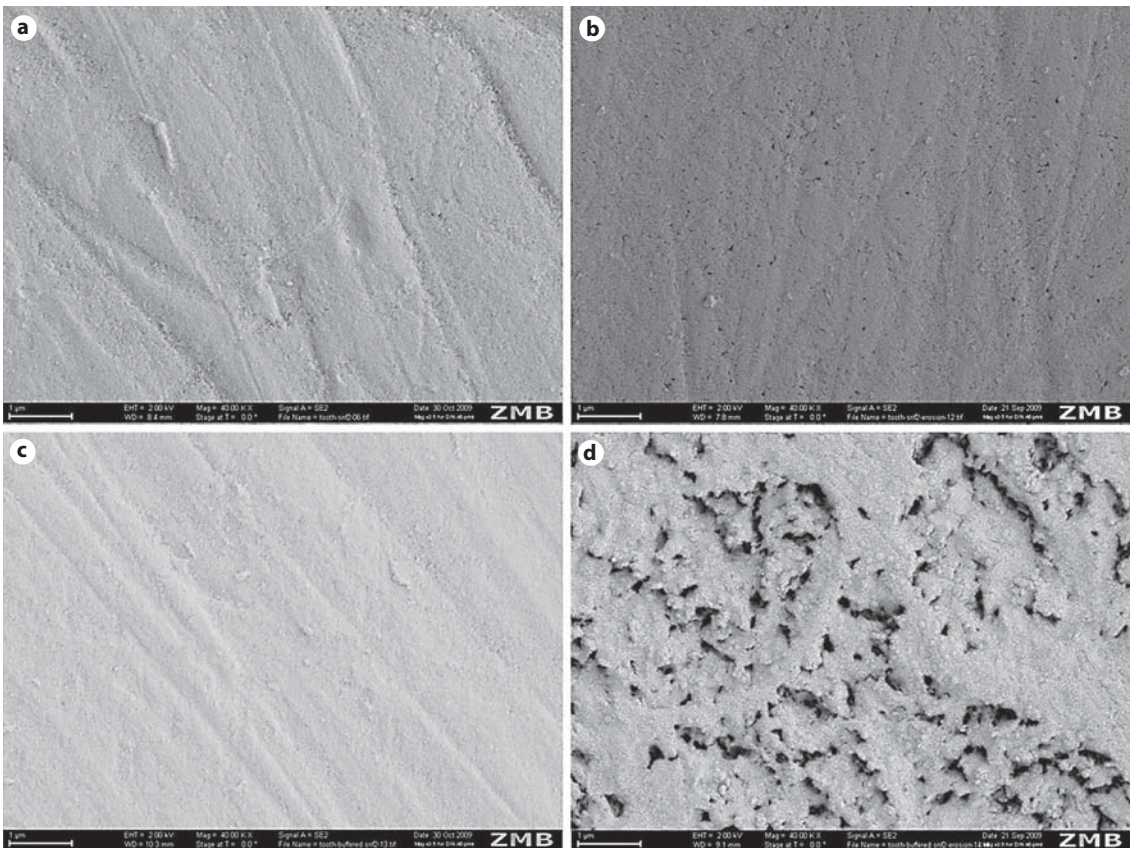

Fig. 4. Representative images of enamel treated with SnF_2. **a** SEM image of enamel surface after native SnF_2 (pH 2.7) application. ×40,000. **b** SEM image of native SnF_2-treated enamel surface after erosion. ×40,000. **c** SEM image of enamel surface after buffered SnF_2 (pH 4) application. ×40,000. **d** SEM image of buffered SnF_2-treated enamel surface after erosion. ×40,000.

2000], globular calcium fluoride (CaF_2)-like globular precipitates were found on the enamel surface after application of AmF at pH 4.6 and 4. The appearance of the precipitates was found to be pH-dependent. The treatment of AmF at pH 4.6 resulted in larger globular precipitates and higher F concentration on the surface than at pH 4. Less phosphate was found in the larger precipitates after topical application of AmF at pH 4.6 than at pH 4 (10.43 vs. 14.54 wt%; fig. 2b, f). Similarly, for both native AmF and buffered AmF-treated samples, the P concentration of the precipitates on the surface showed a decrease after erosion (for native AmF-treated, from 10.43 to 9.92 wt%; for buffered AmF-treated from 14.54 to 12.38 wt%). The differences in the appearance of the globular precipitates seem to be related to phosphate contamination [Rolla and Saxegaard, 1990]. After erosion, a distinct surface alteration with larger globular precipitates of CaF_2-like deposits was found in both AmF-treated surfaces. It is well known that the solubility of CaF_2-like deposit decreases with an increase in size of the globules [Nelson et al., 1983]. Thus, it can be speculated that the smaller CaF_2-like materials dissolved during demineralization process and the mineral and fluoride tended to fuse into bigger CaF_2-like materials. Additionally, the surfactant properties of AmF might contribute to its erosion-prohibiting potential [Arweiler et al., 2003]. Interestingly, some polishing traces on the native and buffered AmF-treated samples were still visible after erosion, correlating well with the profilometric data and corroborating the protective effect of the AmF. However, it is noteworthy that the thickness of the analyzed layer is a few microns under the usual conditions of EDS analysis [Verita et al., 1994]. As seen from the figures 1–3, the globular precipitates varied in size on the enamel surfaces. Therefore, the EDS spectra might contain the signal not only from the precipitates, but also from the underlying enamel.

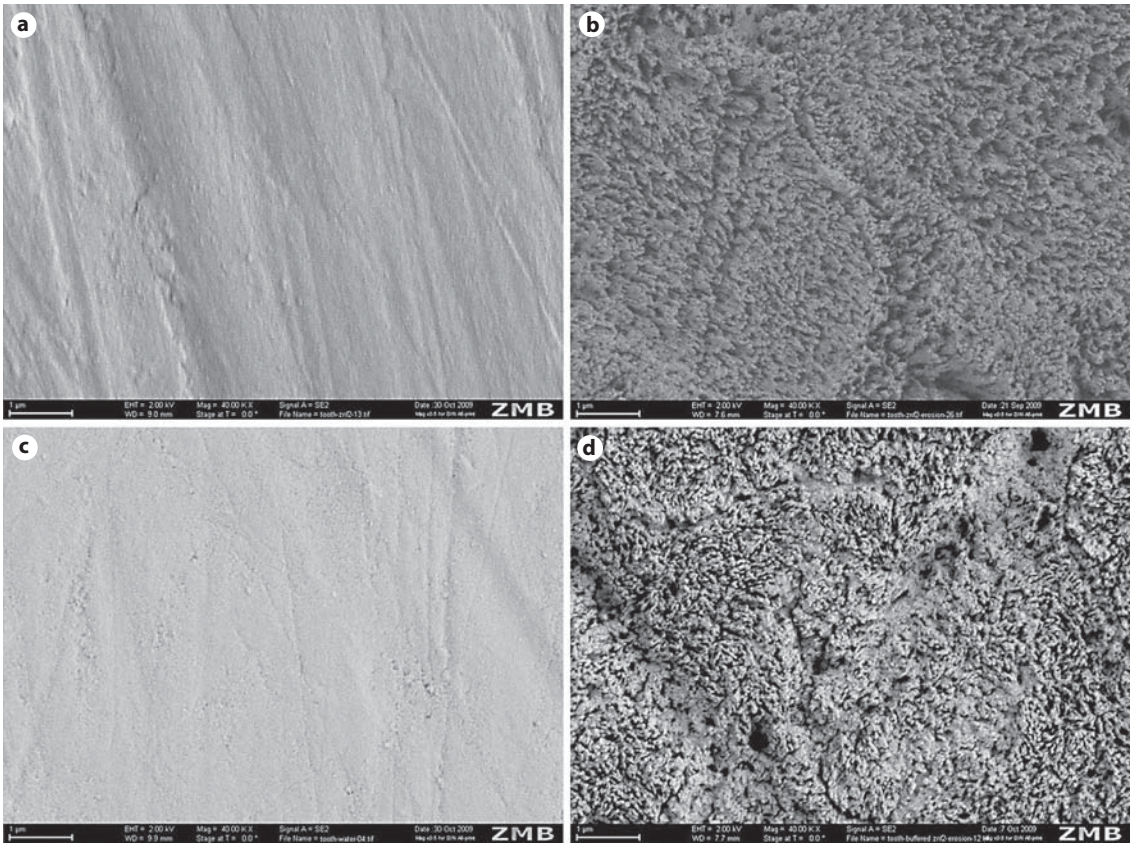

Fig. 5. Representative images of enamel treated with ZnF_2. **a** SEM image of enamel surface after native ZnF_2 (pH 5.1) application. ×40,000. **b** SEM image of native ZnF_2-treated enamel surface after erosion. ×40,000. **c** SEM image of enamel surface after buffered ZnF_2 (pH 4) application. ×40,000. **d** SEM image of buffered ZnF_2-treated enamel surface after erosion. ×40,000.

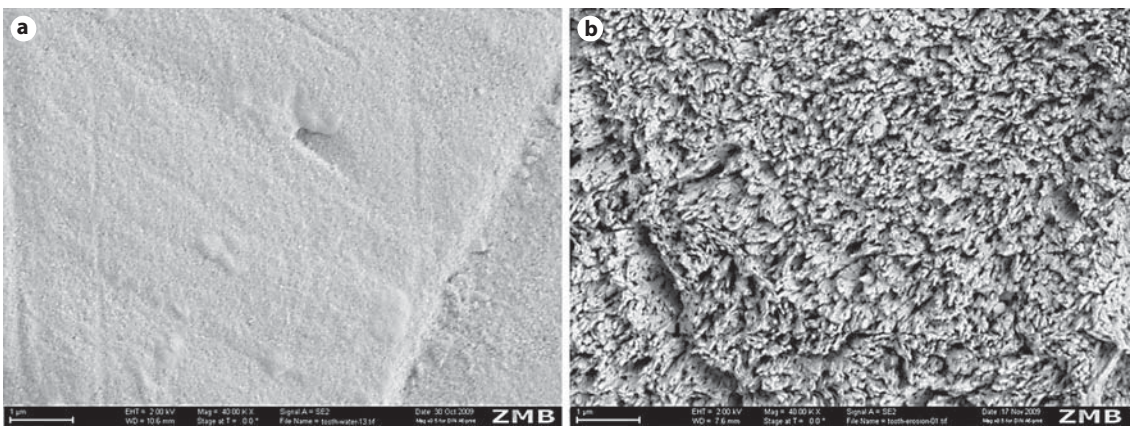

Fig. 6. Representative images of enamel treated with distilled water (control). **a** SEM image of enamel surface after distilled water application. ×40,000. **b** SEM image of control enamel surface after erosion. ×40,000.

The application of native TiF_4 resulted in minor enamel loss (0.25 μm), which was much lower than the application of fluoride-free buffer solution at the same pH (3.17 μm). Moreover, this application led to a build-up of a layer on the enamel surface containing Ti, which was in accordance with previous studies [Magalhaes et al., 2008; Wiegand et al., 2009b]. The mechanism of layer formation following application of TiF_4 is still not clear. It is likely that within a dissolution-precipitation process a new compound (hydrated hydrogen titanium phosphate) is formed [Ribeiro et al., 2006]. During the application, calcium in the hydroxyapatite lattice may be replaced by titanium [Leadley et al., 1997]. Further, the SEM images (fig. 1b) showed severely demineralized enamel under the surface coating, suggesting that enamel might suffer from a dissolution-precipitation process after the application of native TiF_4 solution. It was reported that the application of TiF_4 at pH 1.2 led to a dense surface layer [Wiegand et al., 2009b]. However, in the present study, the layer was found to have microcracks most likely due to specimen preparation prior to EDS. The development of cracks during desiccation may be an indication of a gel-like composition of this layer. Apart from Ti, the surface F concentration increased after native TiF_4 treatment. Therefore, we may assume that some fluoride-containing compounds are also formed apart from titanium phosphate. The composition of the globular deposits, having higher F and Ti concentration and lower Ca concentration compared with the surface layer (fig. 1c, d), can add some support to this hypothesis. Contrasting to the results of the previous study [Wiegand et al., 2009b], the formed layer was partially dissolved after erosion. This might be due to the more intensive erosive attacks adopted in the present study (total of 30 vs. 10 min acid challenge). This Ti-rich layer did provide a protective barrier to the enamel surface capable of providing protection against erosive attack for a certain period of time as shown by profilometry. Furthermore, it is noteworthy that the application of TiF_4 at pH 4 failed to form this Ti-rich coating on the enamel surface, thus leading to a similarly high enamel loss as the control group after erosion.

Both native SnF_2 and buffered SnF_2 were able to reduce erosive enamel loss. The application of SnF_2 resulted in an increase of Sn, indicating the possible reaction between Sn and the HAP lattice [Hove et al., 2008]. Since there was only a minor increase in the F concentration on the SnF_2-treated surface, the protective effect of the SnF_2 solution might be largely due to the Sn-containing reaction products [possibly Sn_2OHPO_4, $Sn_3F_3PO_4$, and $Ca(SnF_3)_2$] [Babcock et al., 1978]. However, in contrast to previous studies [Wei, 1974; Ganss et al., 2008], the so-called Sn-rich coating was not found after application of SnF_2. A possible explanation could be the different tin concentration and application time of SnF_2 solution used in this study.

After erosion, all samples showed a reduced surface Ca concentration, indicating that demineralization happened on the enamel surface due to erosion. In addition, an interesting change in surface fluoride concentration should be noted. After application of native TiF_4, native and buffered AmF, buffered ZnF_2, and buffered NaF, the F concentration of the enamel surface increased. This phenomenon was probably related to the formation of CaF_2-like materials on the surface, although some of the changes were not detectable under SEM. After erosion, the surface F concentration was reduced, possibly indicating the dissolution of the CaF_2-like precipitation. Interestingly, for the samples treated with buffered AmF, the surface fluoride concentration remained stable after erosion, although a distinct difference was found using SEM. Nevertheless, further studies are needed to clarify this issue.

In accordance with a previous study [Schlueter et al., 2009], NaF and ZnF_2 were not able to reduce erosive enamel loss, although the application of buffered NaF and ZnF_2 led to the distinct formation of CaF_2-like materials on the surfaces. Possibly, the CaF_2-like precipitates formed by the application of buffered NaF and ZnF_2 dissolved quickly in the beginning of the acid attack. This hypothesis could be confirmed by the fact that the substance loss of buffered NaF- and ZnF_2-treated samples were only a bit lower than in the control samples.

A further point to consider is that the pH of fluoride solution plays an important role in the efficacy of fluoride agents against erosion. Based on the findings of this study, the fluoride solutions at lower pH increased the surface F concentration (except for AmF) and provided better protection against erosive enamel loss. This phenomenon could be partially explained by the increased formation of CaF_2-like deposit [ten Cate, 1997] and better incorporation of metal ions (Ti and Sn) into enamel under lower pH condition.

Due to the low solubility of $ZnF_2 \cdot 4H_2O$ (1.6 g/100 g water) [International Union of Pure and Applied Chemistry, 2009], the fluoride concentration of ZnF_2 solution was 0.20 M. Since there is some evidence that the protective effects of fluoride agents were not strongly dependent on the fluoride concentration [Wegehaupt et al., 2009; Wiegand et al., 2009a], the relatively low fluoride concentration of ZnF_2 may not be a critical problem in this study.

In this study, various high-concentrated fluoride agents were tested and the distinct protective effects of TiF_4, AmF and SnF_2 application on the development of erosive lesions were shown. However, further in situ or clinical studies are needed to see whether it is valid to recommend high-concentrated fluoride application for prevention of dental erosion.

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References

Arnold WH, Haase A, Hacklaender J, Gintner Z, Banoczy J, Gaengler P: Effect of pH of amine fluoride containing toothpastes on enamel remineralization in vitro. BMC Oral Health 2007;7:14.

Arweiler NB, Auschill TM, Baguley N, Netuschil L, Sculean A: Efficacy of an amine fluoride-triclosan mouthrinse as compared to the individual active ingredients. J Clin Periodontol 2003;30:192–196.

Attin T: Methods for assessment of dental erosion. Monogr Oral Sci 2006;20:152–172.

Attin T, Becker K, Roos M, Attin R, Paque F: Impact of storage conditions on profilometry of eroded dental hard tissue. Clin Oral Investig 2009;13:473–478.

Babcock FD, King JC, Jordan TH: The reaction of stannous fluoride and hydroxyapatite. J Dent Res 1978;57:933–938.

Barbour ME, Rees JS: The laboratory assessment of enamel erosion: a review. J Dent 2004;32: 591–602.

Ganss C, Klimek J, Brune V, Schurmann A: Effects of two fluoridation measures on erosion progression in human enamel and dentine in situ. Caries Res 2004;38:561–566.

Ganss C, Lussi A, Scharmann I, Weigelt T, Hardt M, Klimek J, Schlueter N: Comparison of calcium analysis, longitudinal microradiography and profilometry for the quantitative assessment of erosion in dentine. Caries Res 2009;43:422–429.

Ganss C, Schlueter N, Hardt M, Schattenberg P, Klimek J: Effect of fluoride compounds on enamel erosion in vitro: a comparison of amine, sodium and stannous fluoride. Caries Res 2008;42:2–7.

Hove L, Holme B, Ogaard B, Willumsen T, Tveit AB: The protective effect of TiF4, SnF2 and NaF on erosion of enamel by hydrochloric acid in vitro measured by white light interferometry. Caries Res 2006;40:440–443.

Hove LH, Holme B, Young A, Tveit AB: The protective effect of TiF4, SnF2 and NaF against erosion-like lesions in situ. Caries Res 2008; 42:68–72.

International Union of Pure and Applied Chemistry: IUPAC-NIST Solubility Database v. 1.0. 2009. http://srdata.nist.gov/solubility/index.aspx (accessed June 18, 2010).

Jaeggi T, Lussi A: Prevalence, incidence and distribution of erosion. Monogr Oral Sci 2006; 20:44–65.

Klimek J, Hellwig E, Ahrens G: Fluoride taken up by plaque, by the underlying enamel and by clean enamel from three fluoride compounds in vitro. Caries Res 1982;16:156–161.

Kuisma-Kursula P: Accuracy, precision and detection limits of SEM-WDS, SEM-EDS and Pixe in the multi-elemental analysis of medieval glass. X-Ray Spectrom 2000;29:111–118.

Lagerweij MD, Buchalla W, Kohnke S, Becker K, Lennon AM, Attin T: Prevention of erosion and abrasion by a high fluoride concentration gel applied at high frequencies. Caries Res 2006;40:148–153.

Leadley SR, Davies MC, Ribeiro CC, Barbosa MA, Paul AJ, Watts JF: Investigation of the dissolution of the bioceramic hydroxyapatite in the presence of titanium ions using ToF-SIMS and XPS. Biomaterials 1997;18:311–316.

Lussi A, Jaeggi T, Zero D: The role of diet in the aetiology of dental erosion. Caries Res 2004; 38(suppl 1):34–44.

Magalhaes AC, Kato MT, Rios D, Wiegand A, Attin T, Buzalaf MA: The effect of an experimental 4% Tif_4 varnish compared to NaF varnishes and 4% TiF_4 solution on dental erosion in vitro. Caries Res 2008;42:269–274.

Magalhaes AC, Wiegand A, Rios D, Honorio HM, Buzalaf MA: Insights into preventive measures for dental erosion. J Appl Oral Sci 2009;17:75–86.

Nelson DG, Jongebloed WL, Arends J: Morphology of enamel surfaces treated with topical fluoride agents: SEM considerations. J Dent Res 1983;62:1201–1208.

Paradella TC, Koga-Ito CY, Jorge AO: Ability of different restorative materials to prevent in situ secondary caries: analysis by polarized light-microscopy and energy-dispersive X-ray. Eur J Oral Sci 2008;116:375–380.

Ribeiro CC, Gibson I, Barbosa MA: The uptake of titanium ions by hydroxyapatite particles – structural changes and possible mechanisms. Biomaterials 2006;27:1749–1761.

Rolla G, Saxegaard E: Critical evaluation of the composition and use of topical fluorides, with emphasis on the role of calcium fluoride in caries inhibition. J Dent Res 1990;69(spec No):780–785; discussion 820–823.

Rosin-Grget K, Lincir I, Tudja M: Effect of amine fluoride on enamel surface morphology. Coll Antropol 2000;24:501–508.

Schlueter N, Duran A, Klimek J, Ganss C: Investigation of the effect of various fluoride compounds and preparations thereof on erosive tissue loss in enamel in vitro. Caries Res 2009;43:10–16.

Schlueter N, Ganss C, Mueller U, Klimek J: Effect of titanium tetrafluoride and sodium fluoride on erosion progression in enamel and dentine in vitro. Caries Res 2007;41:141–145.

ten Cate JM: Review on fluoride, with special emphasis on calcium fluoride mechanisms in caries prevention. Eur J Oral Sci 1997;105: 461–465.

Verita M, Basso R, Wypyski MT, Koestler RJ: X-ray microanalysis of ancient glassy materials: a comparative study of wavelength dispersive and energy dispersive techniques. Archaeometry 1994;36:241–251.

Vieira A, Ruben JL, Huysmans MC: Effect of titanium tetrafluoride, amine fluoride and fluoride varnish on enamel erosion in vitro. Caries Res 2005;39:371–379.

Wegehaupt FJ, Solt B, Sener B, Wiegand A, Schmidlin PR, Attin T: Influence of fluoride concentration and ethanol pre-treatment on the reduction of the acid susceptibility of enamel. Arch Oral Biol 2009;54:823–829.

Wei SH: Scanning electron microscope study of stannous fluoride-treated enamel surfaces. J Dent Res 1974;53:57–63.

Wiegand A, Bichsel D, Magalhaes AC, Becker K, Attin T: Effect of sodium, amine and stannous fluoride at the same concentration and different pH on in vitro erosion. J Dent 2009a;37:591–595.

Wiegand A, Meier W, Sutter E, Magalhaes AC, Becker K, Roos M, Attin T: Protective effect of different tetrafluorides on erosion of pellicle-free and pellicle-covered enamel and dentine. Caries Res 2008;42:247–254.

Wiegand A, Waldheim E, Sener B, Magalhaes AC, Attin T: Comparison of the effects of TiF_4 and NaF solutions at pH 1.2 and 3.5 on enamel erosion in vitro. Caries Res 2009b;43: 269–277.

Yu H, Wegehaupt FJ, Wiegand A, Roos M, Attin T, Buchalla W: Erosion and abrasion of tooth-colored restorative materials and human enamel. J Dent 2009;37:913–922.